The Response of DNA Damage in Normal Human Cell Populations Locally Irradiated with X-Ray Microbeams of Different Beam Sizes: Basic Study to Clarify the Health Effects of Internal Exposure

X-ray microbeams are very effective for reproducing the situation of local exposure in internal exposure *in vitro*. We investigated the response of DNA damage in normal human cell populations locally irradiated with X-ray microbeams of different beam sizes, and found that even at the same dose, the smaller the field size of X-irradiation on the cell population, the fewer DNA double strand breaks per cell in X-irradiated cells. These results indicate that X-irradiated cells received some signal (rescue signal) from surrounding non-irradiated cells, and DNA damage was rapidly repaired or cells with DNA damage were eliminated.

After the Fukushima Daiichi Nuclear Power Plant accident caused by the Great East Japan Earthquake on March 11, 2011, insoluble radioactive cesium (Cs-137) was released into the atmosphere, and internal exposure due to Cs-137 deposited in the lungs is currently a problem [1]. The International Commission on Radiological Protection (ICRP) assumes that the cancer risk of internal exposure (local exposure in organs) is equivalent to that of external exposure (uniform exposure in organs) if the average absorbed dose in tissues or organs is the same irrespective of heterogeneous distribution (**Fig. 1**) [2]. However, there is no biological evidence for whether the response of individual cells composing an organ differs between internal and external exposure.

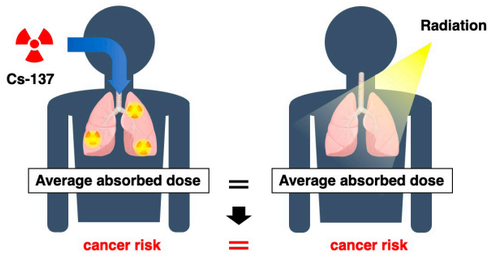

Figure 1: Concept of cancer risks of internal and external exposure by ICRP. ICRP assumes that the cancer risk of internal exposure is equivalent to that of external exposure if the average absorbed dose in tissues or organs is the same.

The X-ray microbeam system in Photon Factory using synchrotron radiation has the advantage of small divergence, which enables us to define the irradiation area precisely by cutting out the X-ray beam [3-7]. Thus, X-ray microbeam irradiation is very effective for reproducing the heterogeneous dose distribution of the cell population following internal exposure *in vitro*.

The present study aimed to clarify the effects of internal exposure by investigating the response of DNA damage in normal human cell populations locally irradiated with X-ray microbeams of different beam sizes.

Normal human fibroblast cells, MRC-5, were cultured on a cover glass to create a cell population of 133 mm^2, and the field sizes of 0.02 mm^2, 0.09 mm^2, 0.81 mm^2, and 1.89 mm^2 on the cell population were irradiated with X-ray microbeams (5.35 keV) of 1 Gy (**Fig. 2**). Subsequently, the numbers of DNA double strand breaks (DSBs) per cell were measured by immunofluorescence staining of 53BP1, which is a DSB repair protein frequently used to detect DSB sites, for up to 48 h post-irradiation.

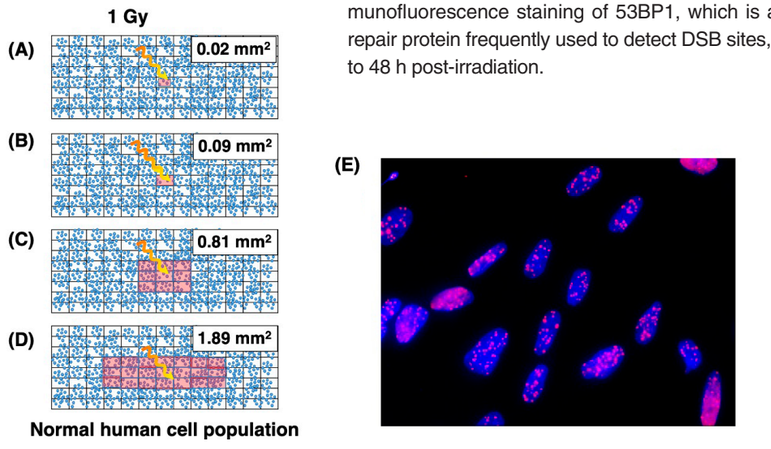

Figure 2: Schematic representation of X-ray microbeams for different field sizes and representative immunofluorescence images of 53BP1 foci. X-ray microbeams were applied at 0.023 mm^2 **(A)**, 0.09 mm^2 **(B)**, 0.81 mm^2 **(C)**, and 1.89 mm^2 **(D)** irradiation field sizes to cell populations by an X-ray microbeam generator. Immunofluorescent image of 53BP1 was observed as DSB **(E)**. Blue: Nuclei, Red: 53BP1 foci.

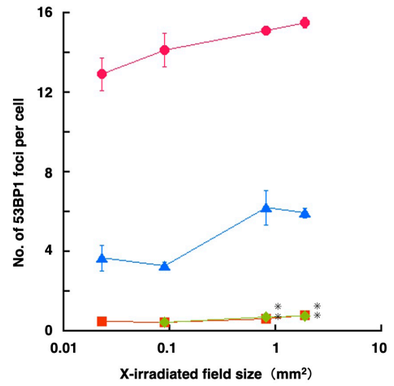

Figure 3: Effects of X-irradiated field size on the DNA damage response. The results are represented as mean ± standard error. Circles, triangles, squares, and rhombi indicate the number of 53BP1 foci per cell of X-irradiated cells in 1 h, 4 h, 24 h, and 48 h post-irradiation, respectively. At 1.89 mm^2 and 0.81 mm^2, the number of 53BP1 foci was significantly higher than background level at 24 h or more after X-irradiation (✳ $p<0.05$, t-test).

The first key finding of this study is that, even at the same dose, the number of DSBs per cell increases depending on the field size of X-irradiation on the cell population (**Fig. 3**). In 1984, Peel *et al.* analyzed the incidence of skin disorders in circular areas of pig skin from 1 to 40 mm in diameter that were irradiated with β-rays. They showed that, even with the same absorbed dose on skin, the larger the β-irradiation field area, the higher the incidence of skin disorders [8]. Similar results have been confirmed by other experimental systems [9, 10]. This phenomenon is called radiation-induced field size effect (RIFSE). Thus, RIFSE was confirmed in this study.

The second key finding is that when the X-irradiated field size is <0.09 mm^2 or lower, the number of DSBs per cell decreases to background levels at 24 hours post-irradiation (**Fig. 3**). In 2011, Chen et al. reported that when human cervical cancer cells were irradiated with 20 cGy of α-rays and co-cultured with non-irradiated human primary fibroblast cells, the number of DSBs in the α-irradiated cells was less than that in α-irradiated cells that were not co-cultured with non-irradiated cells at a statistically significant level. Similarly, micronucleus formation in α-irradiated cells and the number of annexin V-positive apoptotic cells upon α-irradiation were reduced in the presence of non-irradiated cells [11]. This phenomenon is called the radiation-induced rescue effect (RIRE), in which detrimental effects in targeted irradiated cells are reduced upon receiving feedback signals from partnered non-irradiated bystander cells, or from medium previously conditioned with these partnered non-irradiated bystander cells [12]. From these reports, we thought that RIRE may occur in cells at the boundary between the X-irradiated field and the non-irradiated field. Thus, we hypothesized that, in a small irradiation field size, most X-irradiated cells are in contact with non-irradiated cells, which can lead to RIRE, and so RIRE is involved in RIFSE.

The results of the present study suggest that RIFSE is also observed in normal human cell populations by local X-irradiation. The results also provide biological evidence when considering the cancer risks of internal exposure to radionuclides deposited heterogeneously. Furthermore, we speculate that RIRE might be involved in the response of cell populations post-irradiation. This point will be important when considering the concept of dose linked to biological effects such as beam radiation therapy; further research is required.

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BEAMLINE

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